Preliminary Notice upon the Cytology of the Brains of Some Amphibians:

I. NECTURUS.

With Two Plates.

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PRELIMINARY NOTICE UPON THE CYTOLOGY OF THE BRAINS OF SOME AMPHIBIANS: I. NECTURUS.[1]

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With Plates XII and XIII.

In view of the great impulse which has been given to cytological work within recent years, the writer has felt that a study of the cells of the brains of some of the lower animal forms might give a clue to the correct interpretation of results obtained with human material both normal and diseased.

The study of the nerve cells by means of the methylene blue stain (Nissl) has suggested a number of problems with far-reaching results. The past few years have seen a large accession of workers using this method and the question, as the writer sees it, resolves itself into one of interpretation. It is true that the physico-chemical changes taking place in nerve cells are accompanied by constant changes in molar composition as Nissl claims? Can these changes be registered and studied by technical microscopical methods as outlined by Hodge, Vas, Mann and numerous others; and finally is the Nissl method or its modifications such a method, and can its pictures be relied upon to give accurate and trustworthy results? Such a broad outline is, however, manifestly beyond the purpose of the present paper which will simply state some points of possible interest obtained thus far in a research which the writer will hardly be able to complete for the present.

For a number of years it has been known that many cells

[1] The writer wishes to express here his thanks to Professors Osborn and Wilson for their many courtesies and to Dr. Strong for his aid and advice while working in the laboratory.

in the central nervous system of vertebrates, especially the motor cells in the ventral columns of the cord and those in the nuclei of origin of motor nerves, possess a characteristic structure in the presence of what has been termed the chromophilic granules. In general these granules may be described as elongated rod-like, tetrahedronal or irregular portions of protoplasm apparently situated in all parts of the cytoplasm, including the dendrites. In the majority of the cases they seem to occupy the periphery of the cell (cytoplasm) and appear to be arranged in no definite way save perhaps somewhat elongated in the direction of a contiguous dendrite or circularly about the nucleus.

These granules possess marked chemical affinities for certain dyes of the indo-phenol series, notably, methylene, thionine and toluiden blue, though they can be demonstrated by a number of coloring agents. A great variety in the number, shape, size and arrangement has already been described and it becomes a matter of importance to arrive at some definite conceptions regarding them or the cells in which they are found.

The phylogenetic mode of approach has seemed the one which might give the truest answer to this question and the brains of the Amphibia have first been chosen for study. Menopoma, Diemyctylus and Rana have been studied casually, but Necturus was chosen as the basis of the following outline.

If this animal represents a retrograde form in a permanent larval condition, it should give pictures of extreme simplicity and the origins of some of the higher types of nerve cells might be here found.

METHODS.

It may be a matter of tedious annoyance to have to detail the methods by which any results have been attained, yet such is the great amount of technical refinement that it is necessary.

Several preliminary experiments were made with the view of determining the action of different fixing agents. These experiments were first made upon the cord of Necturus, but as this was so small the cord of the cat was finally chosen. Immediately after death the cord was laid bare and a small piece

of uniform calibre was taken from the cervical region. This was cut into seven nearly equal segments and immediately placed in the following hardening fluids:

(1) Sat. alcoholic solut. corrosive sublimate.
(2) Absolute alcohol.
(3) Alcohol 95%.
(4) Alcohol 95%, formalin 20%, equal vols.
(5) Formalin 20%.
(6) Hermann's fluid.
(7) Flemming's fluid.

In (1), (6) and (7) the pieces remained one hour; in the others, 24.

The pieces were properly identified and then run up into paraffine and blocked, all passing through exactly the same after-treatment in the same vessel, all blocked in the same block, cut at the same stroke of the knife and transferred to the slide, fixed (Mayer) and stained upon the slide. Thus the technique in each series was uniform and similar, excepting the hardening fluid used and the length of time of hardening.

Staining was done by a variety of methods and numerous modifications of the Nissl method were tried. Finally the specimens were mounted in different media, on one slide five specimens of Necturus cord were stained under the same conditions and mounted in different media: Xylol Balsam, Xylol Damar, Xylol Colophonium, Nissl's Benzine Colophonium, Chloroform Styrax.

The tedium of technique approaches its zenith when one takes up the question of staining. An investigator may say "Nissl's" method, but which one is meant? Nissl has described so many that it is hard to know which is followed and although the variations are slight they are of importance to the original describer at least, and who should be more competent to judge? Hence it may be said that in the experiments made all of Nissl's methods, Rehms' modification, etc., were tried, including the latest described at the time of publication. The method giving apparently the most uniform and clear results, was, substantially, staining in warm ½% aq. solut. methylene blue for from 15 to 20

minutes, decolorizing by Nissl's decolorizer or by 95% and absolute alcohol which gave precisely the same pictures.

Thionine blue as recommended by Lenhossék was tried and, while it stains very intensely, the writer found it difficult to deal with.

These experiments seem to show the following:

1. With reference to gross changes due to fixing agents: In the illustrations, six cross sections are figured, which show for themselves. As far as could be made out, absolute alcohol, 95% alcohol and alcohol sublimate acted in almost exactly the same manner; distortion and contraction were great and seemingly evenly distributed in the grey and the white matter. With equal volumes of 20% formalin and 95% alcohol, the distortion and contraction was much lessened, while formalin 20% was almost like the normal cord. With Hermann's and Flemming's solutions, the contraction was extreme, the gray matter seemingly more strongly acted upon. Thus if external form alone would be the guide, formalin 20% gives the best preservation.

2. In looking upon the effect upon the ganglion cells and their minute structure, a different state of things is shown, the correct interpretation of which would seem to be an extremely difficult matter. In the first place, one can not get the body of the same ganglion cell under these various conditions, which, perhaps in a hypercritical sense, vitiates the results; then the various angles at which a cell may be cut should be taken into account; but taking the ganglion cells as they run and choosing those of the larger so-called motor type of the ventral horns, the following conclusions seem warranted by the experiments made.

Between alcohol 95%, and sat. alcoholic sol. of corros. sub. I could detect no essential points of difference. With absol. alcohol, the differences made out were very slight, the pictures being of greater definiteness and sharpness than with the former two reagents. In all three there is contraction of the body of the ganglion cells but as the rest of the cord contracts about

in the same ratio there are no empty spaces left about the cell body.

In the specimens fixed with alcohol-formalin one is struck at first sight by the variation in the staining, these sections taking up methylene blue as well as thionine blue quite strongly, more so than the preceding. In the ganglion cells the body is separated from the surrounding structures slightly; whether this is due to its contraction or the contraction of the surrounding tissues away from it, could not be definitely determined. The granules are slightly indistinct, poorer than with specimens preserved with absolute alcohol. With formalin 20% the staining was even more intense than with the preceding and the contraction of or away from the ganglion cell body was marked. Yet the chromophilic granules came out distinctly. Fixation with Hermann's fluid gave the worst results. The contraction was uniform throughout but the granular structure was irregular, lumpy or broken up into fine granular masses. Flemming's fluid gave better results, the chromophilic granules being often quite sharp and well preserved; contraction was marked, as with the Hermann's.

Thus alcohol 95% and sat. alcoholic sol. of corrosive sublimate gave the sharpest pictures.

As to the use of thionine blue, instead of methylene blue, the comparisons made were in favor of the methylene blue.

With reference to the mounting media, I cannot feel that there is much preference to be shown. Nissl highly recommended benzine colophonium but in the experiments made I could not say that I could detect any differences in the media used, and on submission of the slides to Dr. Strong he could not tell that there was any difference. Perhaps after a lapse of some time there may be differences. Benzine colophonium dries very rapidly and hence may have an advantage in the long run, but after a lapse now of twelve months, the specimens still look alike to my eyes. If I were to express any preference it would be for xylol damar, not on account of its being better but the clearness of the resin is pleasant. Many of my benz. coloph. specimens were of no value at the end of six months. Chlor-

oform styrax from the liquidambar seems a promising medium as it brings out quite sharply the chromophilic granules. Its high refractive index should be born in mind.

DESCRIPTION OF THE CELL STRUCTURES OF NECTURUS.

Spinal Cord.—All of the cells are clustered about the central canal which is bordered by two or three rows of ependyma cells whose nuclei alone are stained. These measure .075 mm. in diameter; nucleus .003 mm. in diameter. Around these cells is an irregular mass of nuclei which collectively approximate a winged appearance. At the angles of the wing, or, if homologous, the horns, especially the ventral horns, there is a collection of larger cells, the nuclei in these being twice the diameter of the others. Some of these are provided with a faint fringe of blue staining cytoplasm about the nucleus but in none of the specimens examined were there any distinct traces of chromophilic granules.

The rest of the nuclei (cells) have about the same diameter and show an intricate reticulate structure, as do those clustered about the central canal.

Medulla.—Passing into the medulla, no changes of note were seen.

At about the level of the glosso-pharyngeal nucleus a number of larger triangular pyramidal cells was seen. These averaged about .035 mm. in diameter, and showed indistinct chromophilic granules of an irregular character, some of which radiated out in the direction of what would appear to be dendrites.

The larger cells making up what appeared to be the motor nucleus of the VII nerve were of much the same character; they averaged .04 mm. and their nuclei were large—.037 mm. In these, faint irregular groupings of stained material approximating the typical chromophilic granules were to be made out.

The motor nucleus of the V nerve showed analogous conditions; here the ganglion cells averaged slightly smaller.

Passing into the *mesencephalon* at about the level of Kingsbury's commiss. optici tecti a number of larger cells were found of peculiar shape. These were more or less square in general ap-

pearance and showed a faint yet distinct protoplasmic covering. They averaged about .03 mm. in diameter, the nucleus being .02 mm. In the cytoplasm were a number of thin streaked masses of colored material quite unlike, however, the typical chromophilic granules. What these "cells" are, I am somewhat at a loss to determine. Throughout their distribution, they remain dorsal and extend slightly laterad being mingled with cells of the regular spinal type. In no cases were any of these cells around the central canal.

The cells of the hypophysis are similar to the regular type; like free nuclei.

Just before reaching the epiphysis there is a sinking in of the cerebral mass, the above mentioned "cells" have practically disappeared, and there was noted the gradual appearance of a dorsal group of large cells in the median line; these vary from .008 to .010 mm.

These do not appear to coalesce with the elliptical ependyma cells which average 17-22 micra in length by 10-16 micra in diameter. At this level the cells around the ventricle are from 8 to 10 rows deep and of the regular spherical free nuclei type, although at rare intervals some cells are seen in which the cytoplasm has responded to the stain, but no granules were found. The cells of the hemispheres were all around the ventricle and were all of the conventional type as were also the cells of the rhinencephalon.

At the time of the final revision the article of Guisseppe Levi, "Ricerche citologiche comparate sulla cellula nervosa dei vertebrati," Rivista di patologia nervosa e mentale, 1897, 5., is received and some of his results were deemed of importance for the present communication. Among Amphibia the author studied Rana and Bufo of the Anura, Triton cristatus, Spelerpes fuscus and Proteus auguinus of the Urodeles; these latter are of more interest from the present point of comparison.

The cells of the ventral columns of the cord have an oval form, the cytoplasm in Spelerpes forming a delicate border about the nucleus. In the Triton this is more distinct and contains chromophilic granules which were sharply cut, sometimes

fused and all lying in the direction of the principal dendrite. The nucleus is oval, has a delicate acidophile membrane and contains a mass of acidophile granules.

Dorsal cells of medulla—The cytoplasm of Triton is sparse. In Spelerpes and Proteus it is not distinguishable; nucleoli are absent in all.

Cells of the Pallium.—No cytoplasm was observed in these cells. The nuclei were oval. The granular olfactory cells were lacking in cytoplasm; the nuclei were oval. In the optic lobes three types of cells were noted: (1) granules similar to those of the olfactory lobes; (2) granules larger than these and richer in basophilic substances; (3) cells with comparatively abundant cytoplasm and containing fused chromophilic granules. These have dark nuclei, which in Triton contain basophilic granules and a homogeneous acidophile substance studded with basophilic granules and in all a nucleolus of the usual character.

Ependyma cells in all contained abundant cytoplasm. The Italian author has not studied these brains from the same point of view as that of the present writer, hence a correlation of the results is somewhat difficult. From the standpoint of cytoplasmic development Spelerpes would represent the most primitive type studied. This would seem to be more primitive even than Necturus, but for the reasons above stated it would be premature to draw any conclusions.

In Necturus we find a predominant type of nerve cells which are indicative of a low grade of development. The chromophilic granules are found in only a few of the cells and when found are elementary or fragmentary in their construction, those of the 7th nucleus appear to be the best developed. The absence of any chromophilic granules in the cells of the pallium is of interest. In Necturus, the chromophilic granules, especially those found in the 7th nerve nucleus, appear to be fibrillar in structure.

Levi's investigations show a similar condition in the pallia of Spelerpes, Triton and Proteus, while in Rana and Bufo a small amount of cytoplasm is found collected in a small conical mass lying to one side of the nucleus, generally the outer side.

The method of staining is, in the author's experience, too restricted, for while it brings out the basophilic structures, the neutrophilic and acidophilic relationships of the different protoplasmic portions of the cell body and nucleus are not shown. Following Flemming, Benda and Levi the compound tinctures might be used to better advantage in the study of the normal nerve cells and further in diseased tissues.

Zoological Laboratory, Columbia University, Jan., 1897.

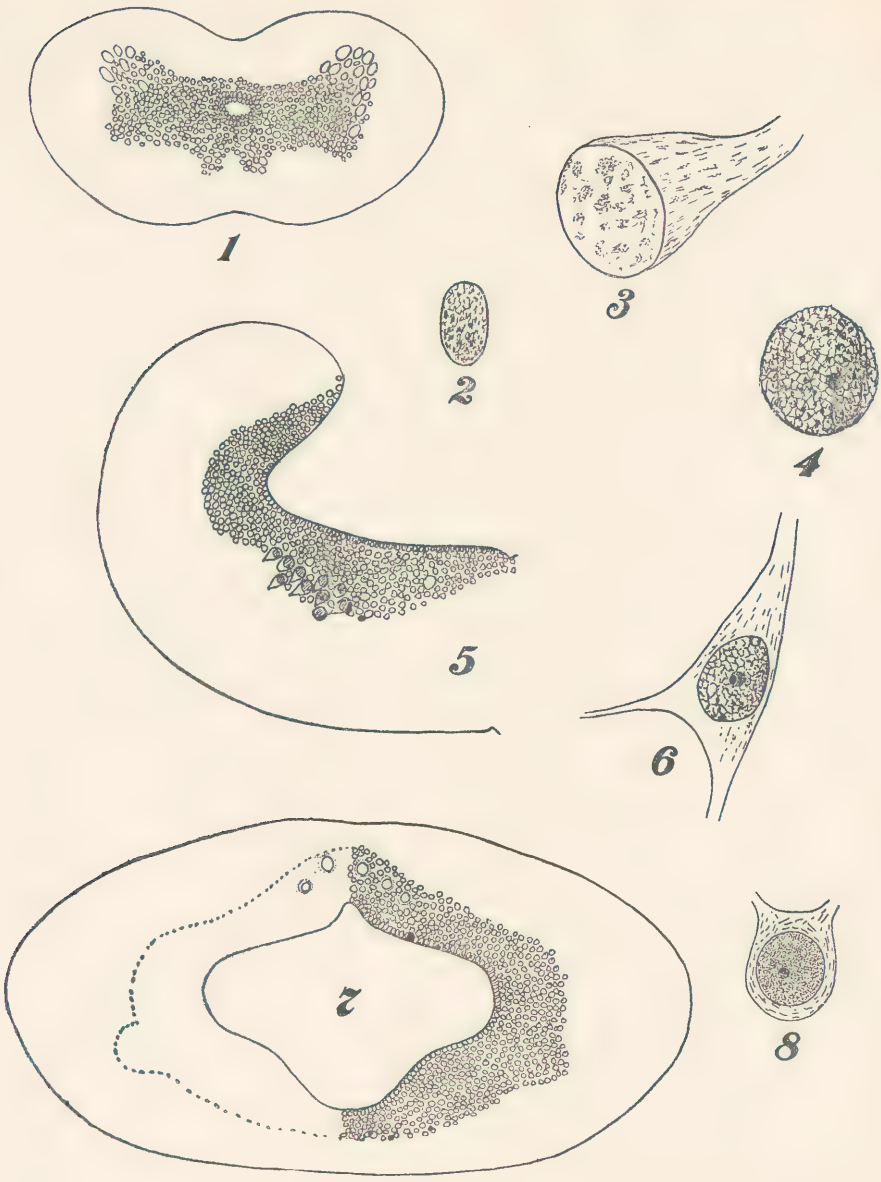
EXPLANATION OF FIGURES.

PLATE XII.

Fig. 1. Cross section of cord of Necturus.
Fig. 2. Nucleus of ependyma cell.
Fig. 3. Cell in cord about level of the 11th or 12th N.
Fig. 4. Nucleus of nerve cells of predominant type.
Fig. 5. Cross section of medulla, 10th N. ?
Fig. 6. Cell of 7th N. nucleus.
Fig. 7. Cross section of mesencephalon.
Fig. 8. Nerve cell of mesencephalon.

PLATE XIII.

Fig. A. Cross section cat's cord. Normal size. Cervical.
Fig. B. Fixation, acohol 95%, formalin 20%; equal parts.
Fig. C. Fixation, alcohol, 95%; also alc. sat. solut. corrosive sublimate.
Fig. D. Fixation, formalin, 20%.
Fig. E. Fixation, absolute alcohol.
Fig. F. Fixation, Flemming's and Hermann's solutions.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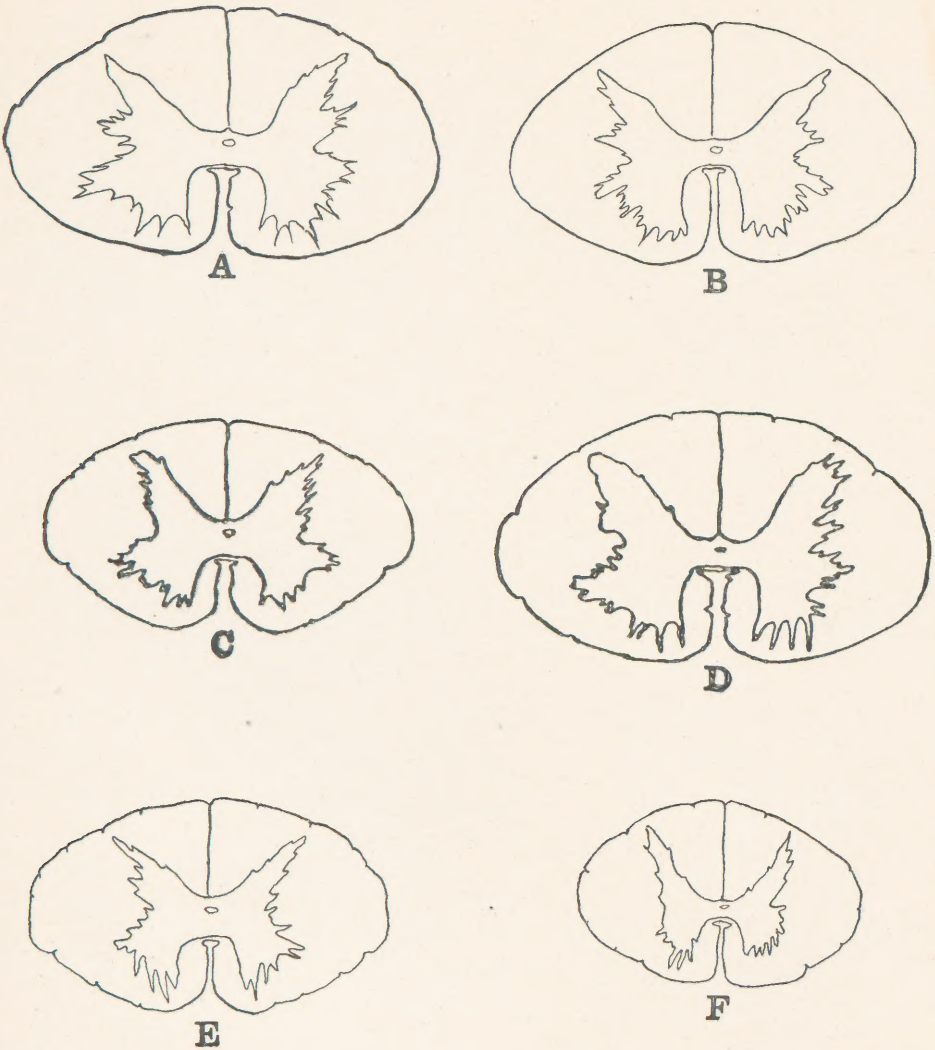
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